The Oral Microflora in Health and Disease

Opportunities for collaboration

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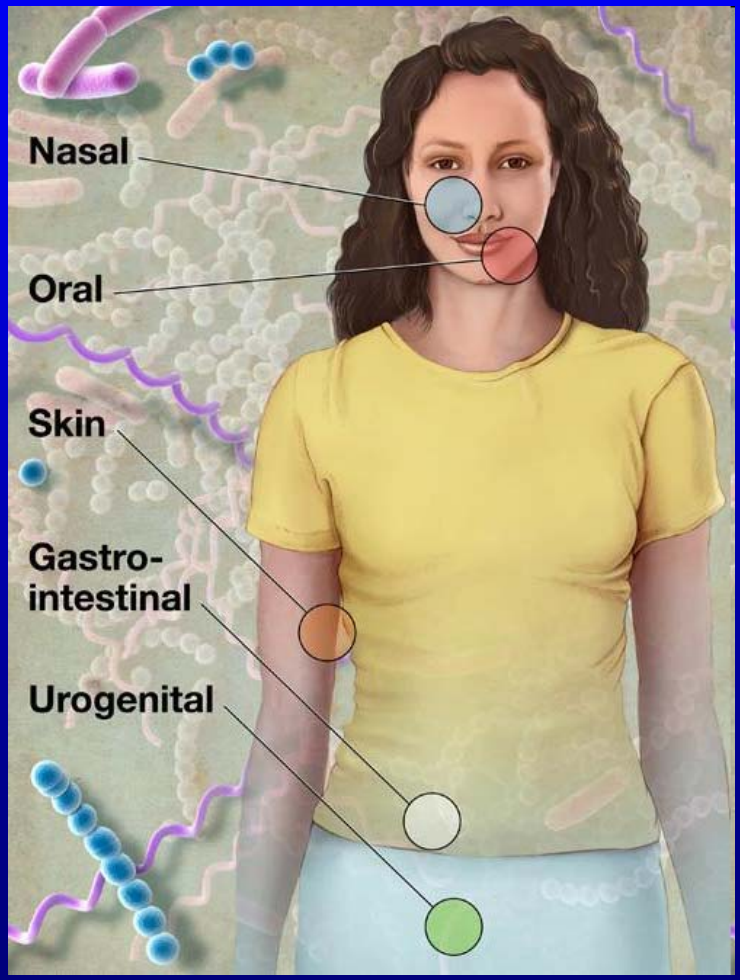
Outline

- Brief introduction to oral microflora
- Some of my work resulting from an SOD/SON collaboration
- Personal examples of other previous SOD/SON collaborations
- Examples of potential future collaborations

Human Microflora

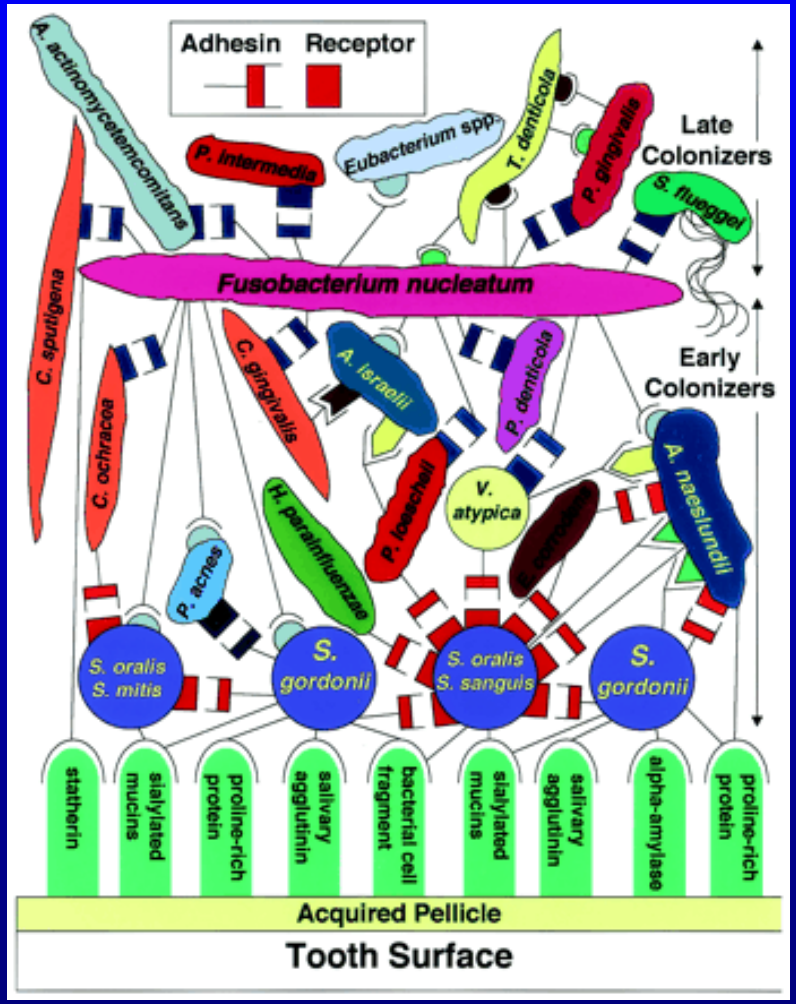
- Based on cell counts, we are 10 parts bacteria and 1 part human
- Based on gene content, we are 150 parts bacteria, and 1 part human



NIH Human Microbiome Project

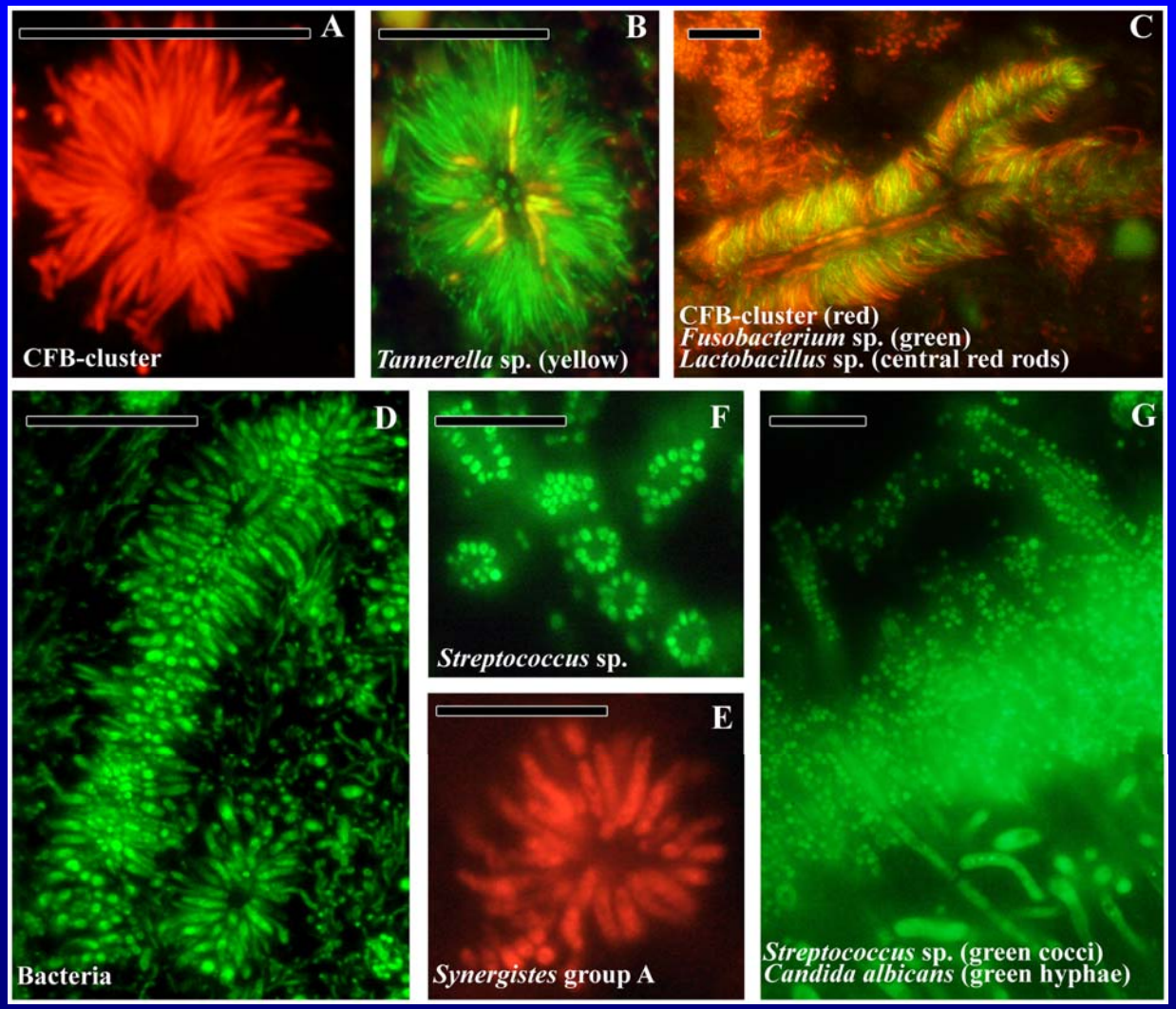
Oral Microflora

- More than 700 species identified
- Composition changes with time and location
- Normal flora likely serves as a barrier to colonization by pathogens



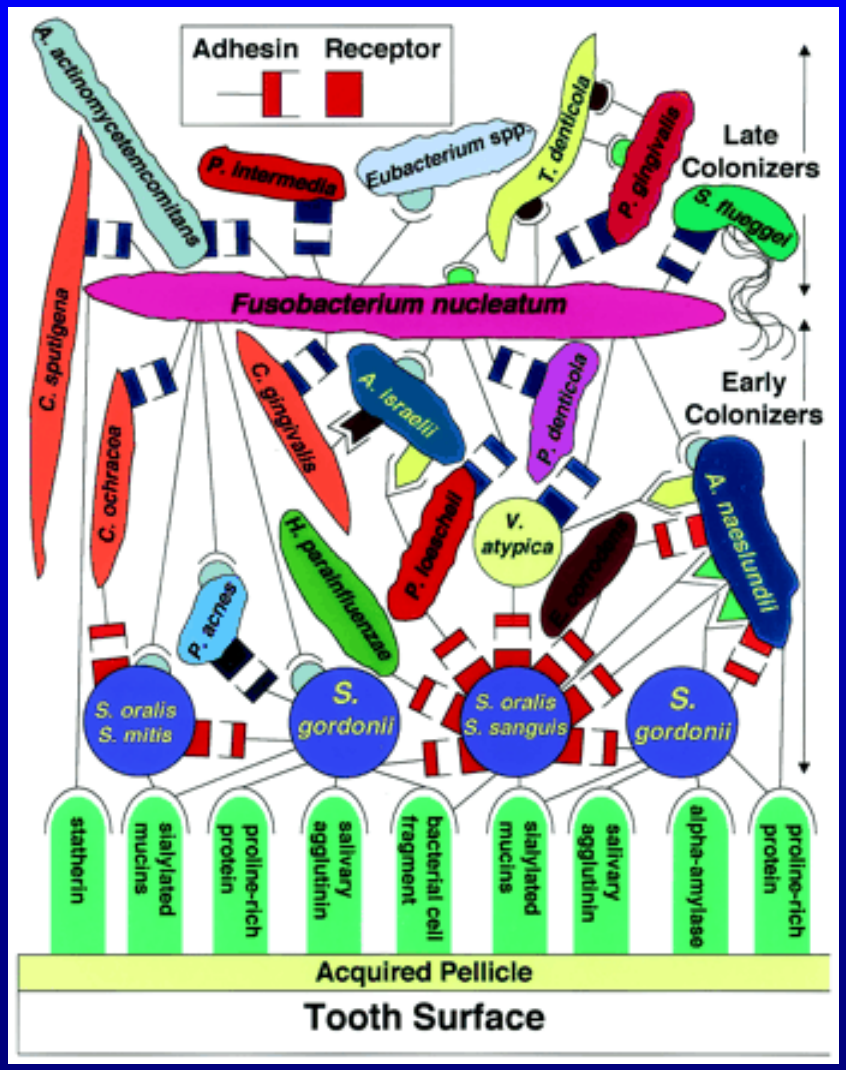
Kolenbrander, P. E., R. N. Andersen, D. S. Blehert, P. G. Egland, J. S. Foster, and R. J. Palmer, Jr. 2002. Communication among oral bacteria. Microbiol Mol Biol Rev **66:**486-505.

Oral Microflora



Zijnge, V., M. B. M. van Leeuwen, J. E. Degener, F. Abbas, T. Thurnheer, R. Gmür, and H. J. M. Harmsen. 2010. Oral biofilm architecture on natural teeth. PLoS ONE **5:**e9321.

Oral Microflora



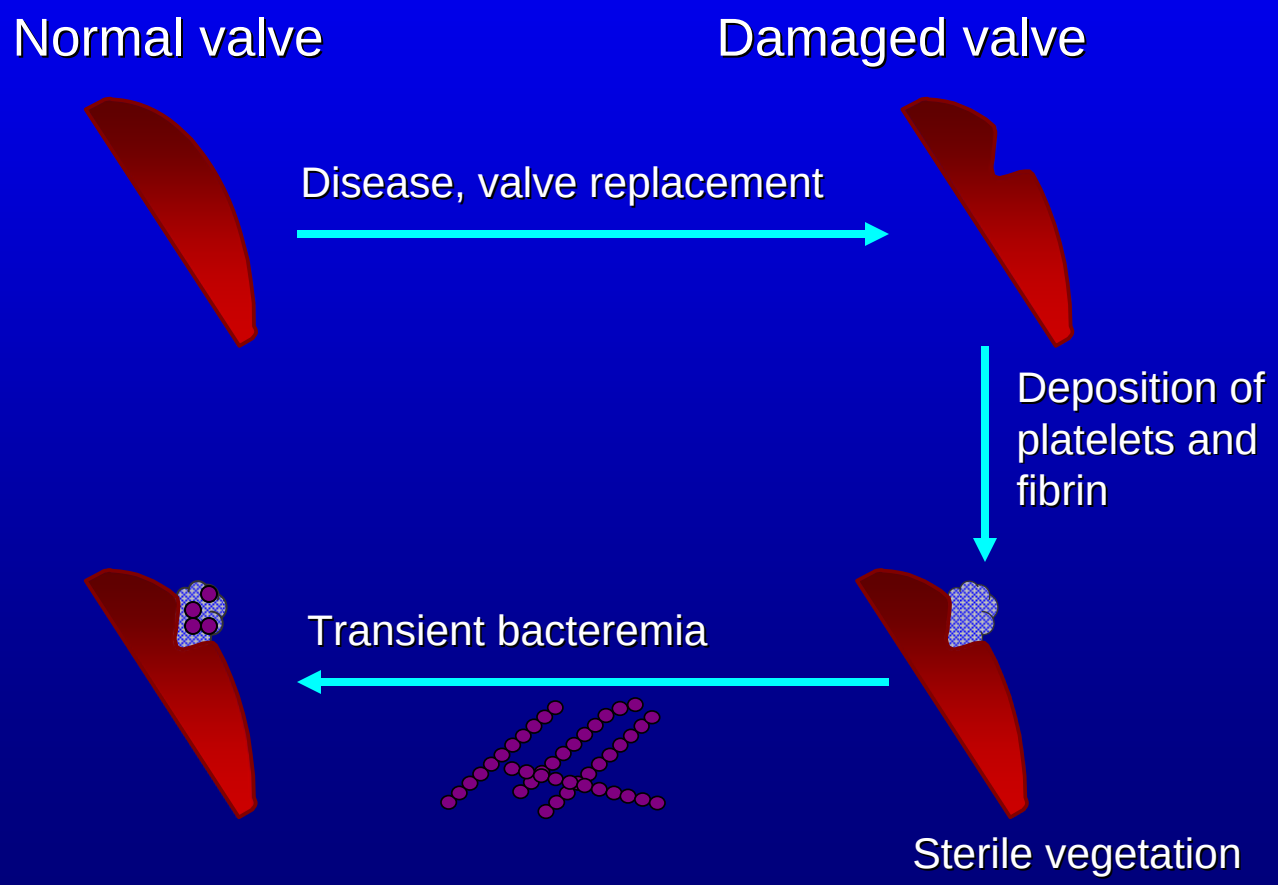
Kolenbrander, P. E., R. N. Andersen, D. S. Blehert, P. G. Egland, J. S. Foster, and R. J. Palmer, Jr. 2002. Communication among oral bacteria. Microbiol Mol Biol Rev **66:**486-505.

Early SOD/SON Collaboration

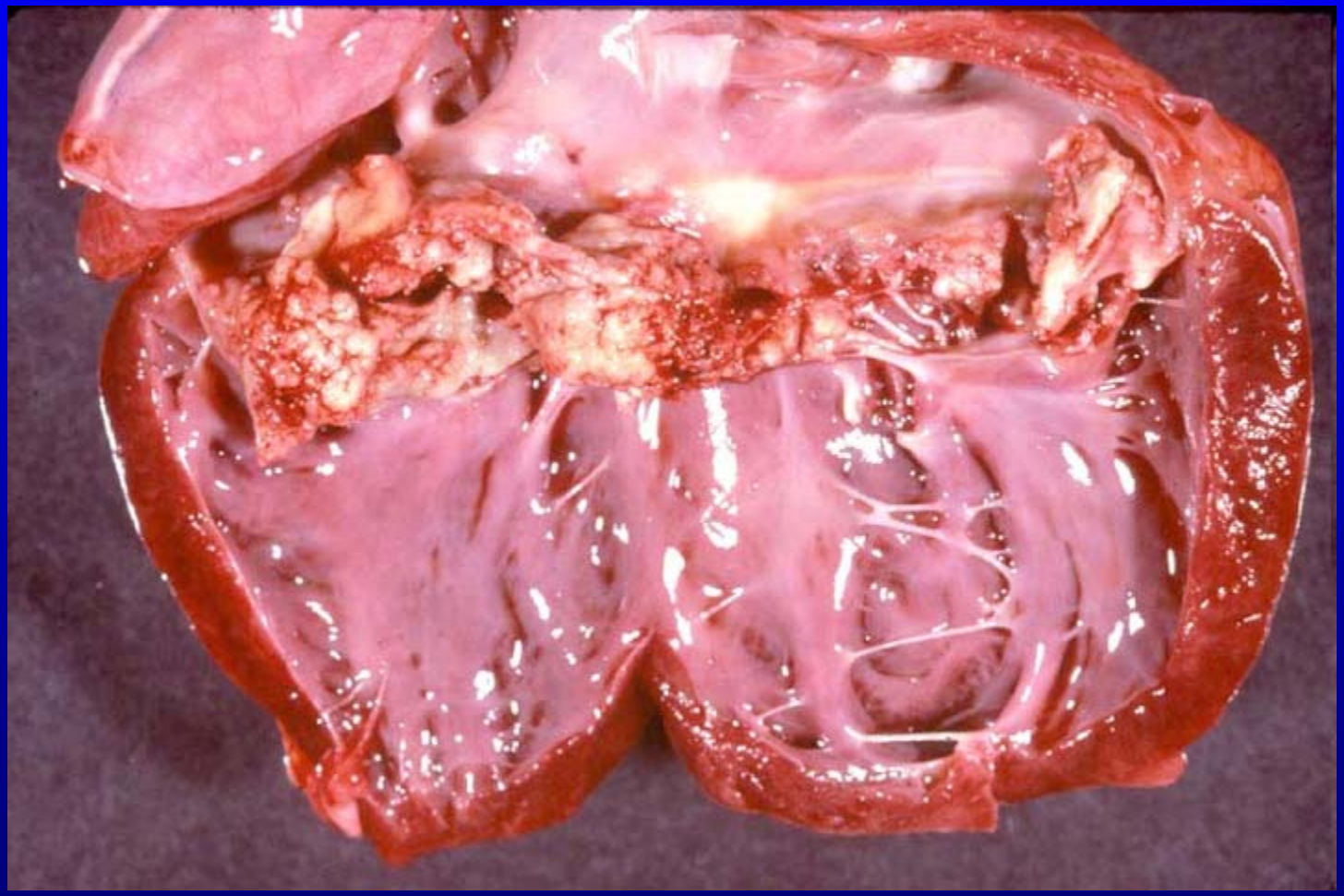
- Frank Macrina: *Streptococcus mutans* and dental caries

- Cindy Munro: *Streptococcus mutans* and infective endocarditis?

Infective Endocarditis Pathogenesis



Infective Endocarditis Pathogenesis



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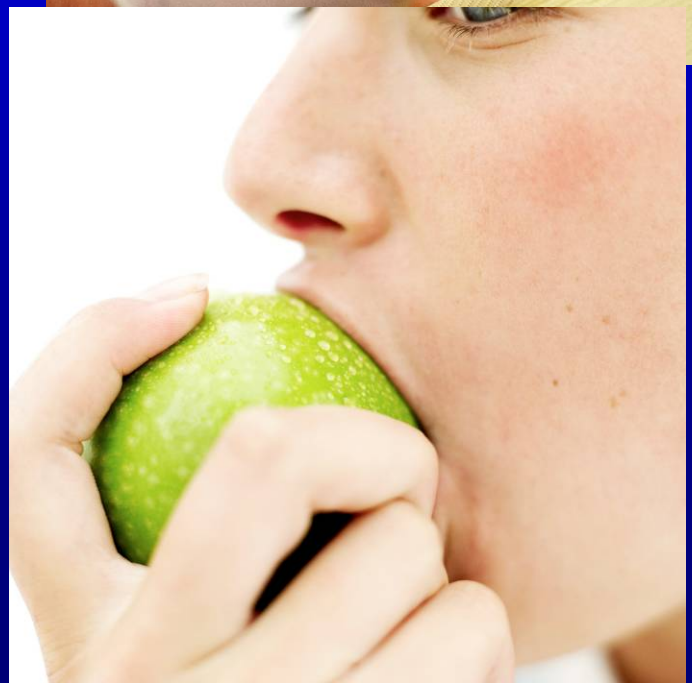
Infective Endocarditis Prevention



Munro and Macrina

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Streptococcus sanguinis genome sequence

SCIENTIFIC BLOGGING
SCIENCE 2.0

Scientists Decode Genome Of Oral Pathogen

By News Staff | April 5th 2007 02:00 AM | Print | E-mail | Track Comments

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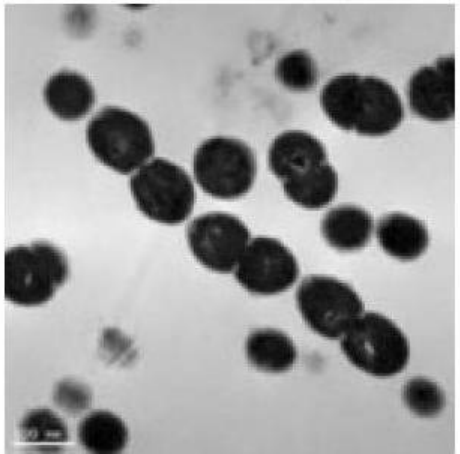
Virginia Commonwealth University researchers have decoded the genome of a bacteria normally present in the healthy human mouth that can cause a deadly heart infection if it enters the bloodstream.

The finding enables scientists to better understand the organism, Streptococcus sanguinis, and develop new strategies for treatment and infection prevention.

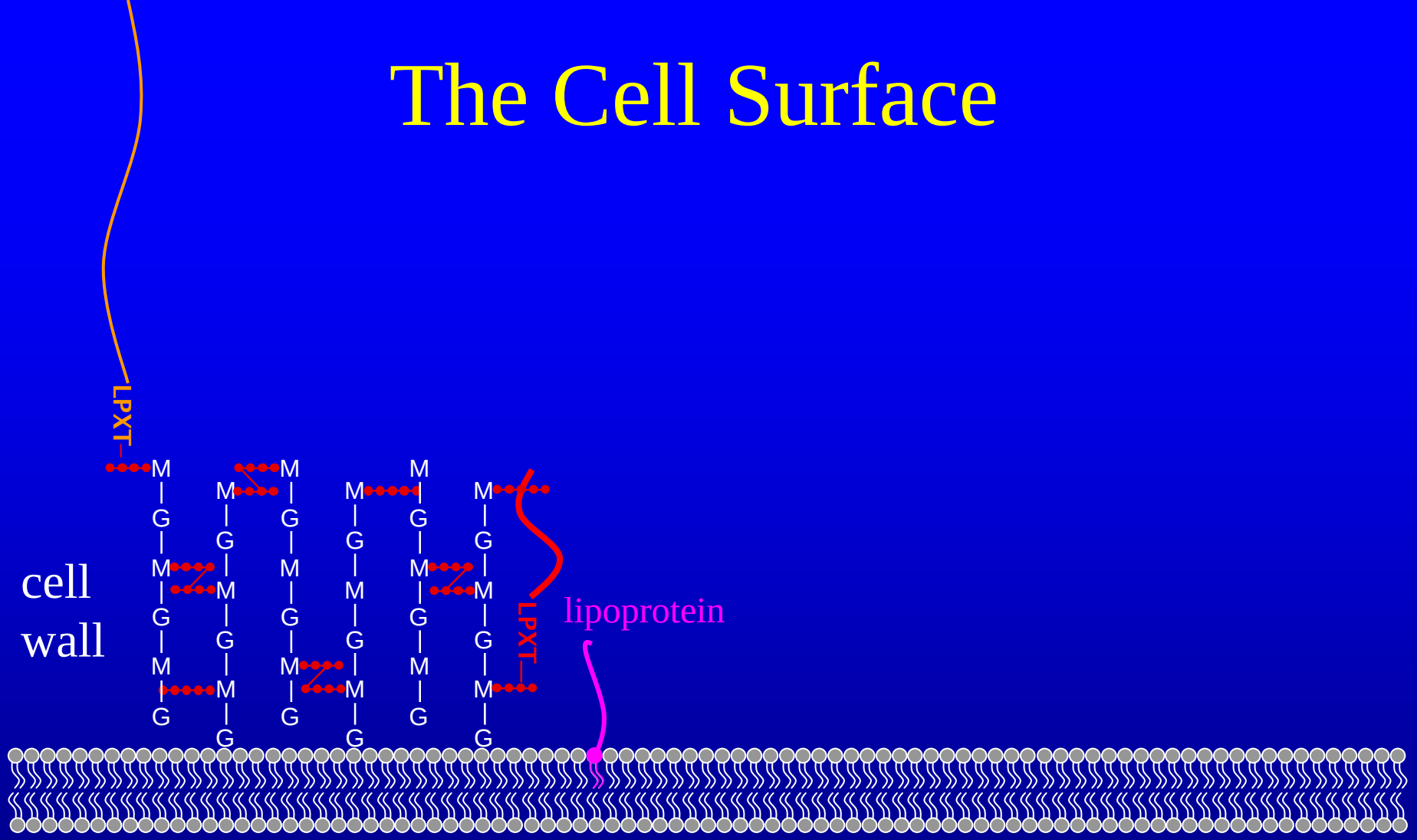


Ping Xu, Ph.D.

- 2.388 Mb
- ~2300 genes

Xu, P., J. M. Alves, T. Kitten, A. Brown, Z. Chen, L. S. Ozaki, P. Manque, X. Ge, M. G. Serrano, D. Puiu, S. Hendricks, Y. Wang, M. D. Chaplin, D. Akan, S. Paik, D. L. Peterson, F. L. Macrina, and G. A. Buck. 2007. Genome of the opportunistic pathogen *Streptococcus sanguinis*. J Bacteriol 189:3166-3175.

The Cell Surface



Lipoprotein candidates

- 53 lipoproteins genes
- Made 53 mutants
- The lipoprotein SsaB was found to be essential for virulence

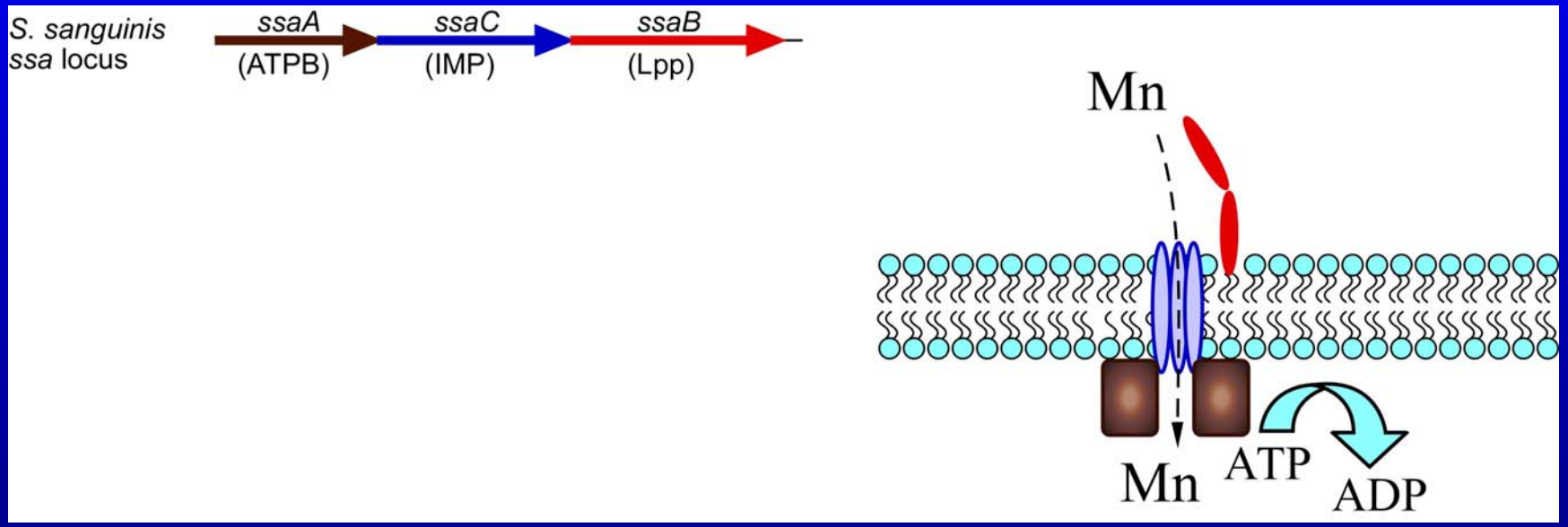
SsaB

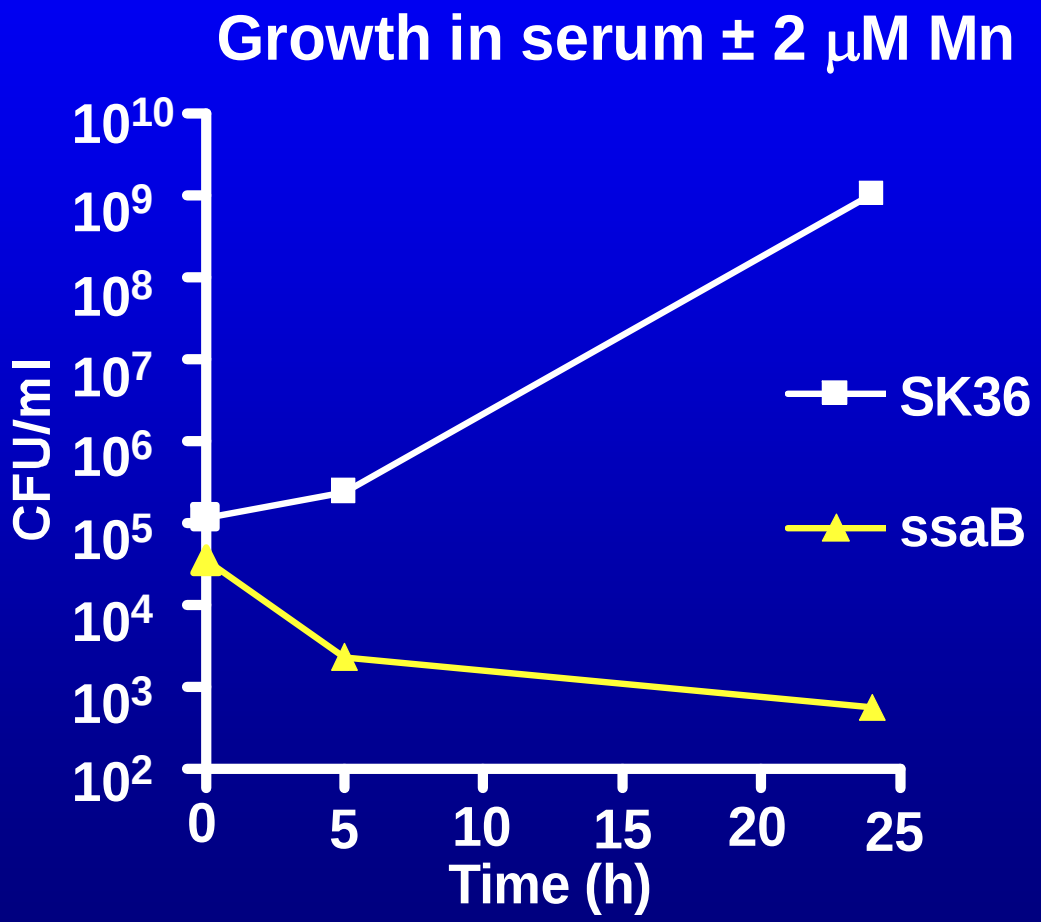
- *S. sanguis* adhesin B

Ganeshkumar, N., M. Song, and B. C. McBride. 1988. Cloning of a *Streptococcus sanguis* adhesin which mediates binding to saliva-coated hydroxyapatite. Infect Immun **56:**1150-1157.

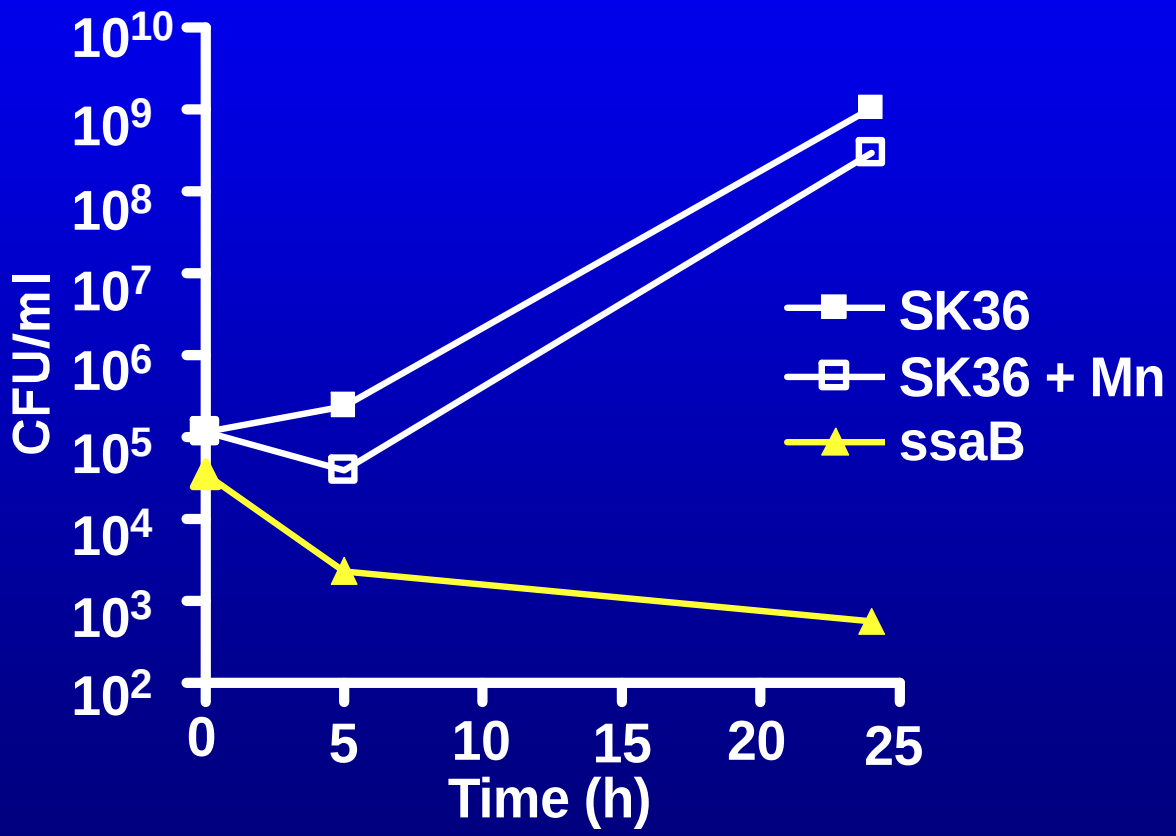
SsaB

- *S. sanguis* adhesin B

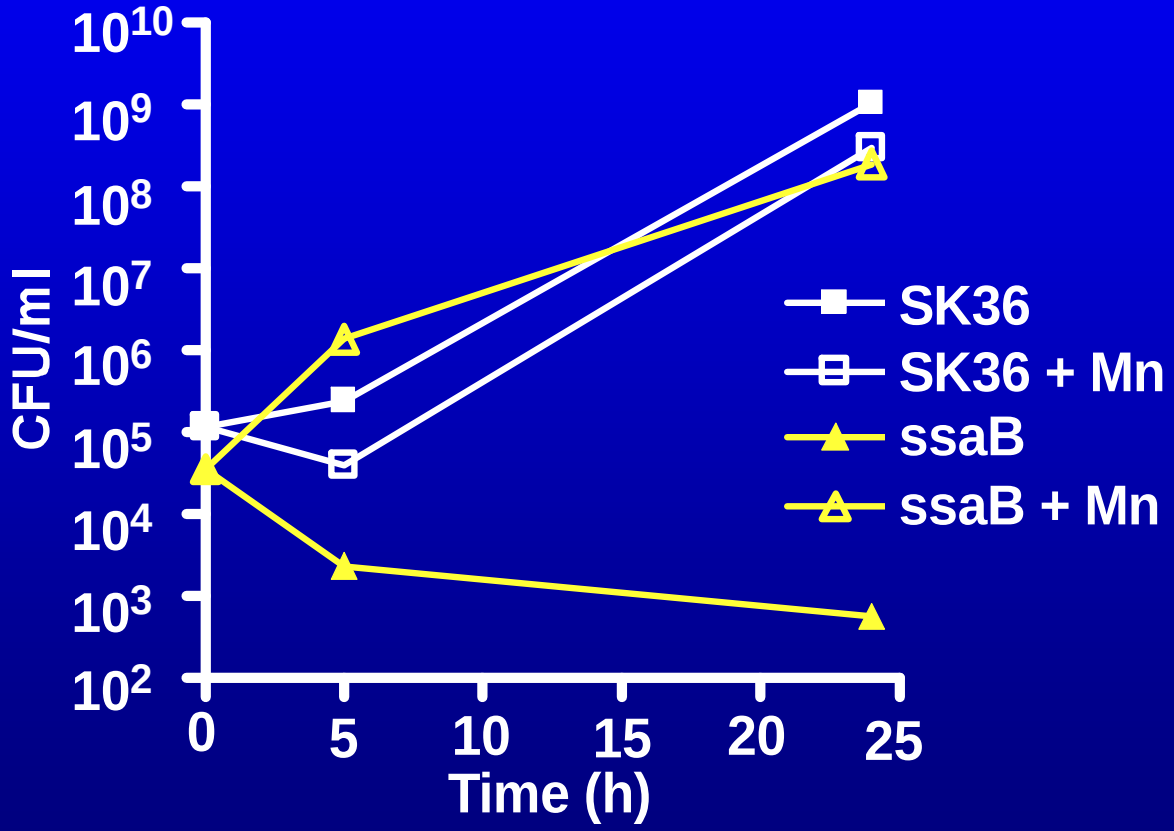



Growth in serum ± 2 μM Mn
CFU/ml
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
5
10
15
20
25
Time (h)
SK36
ssaB

Growth in serum ± 2 μM Mn

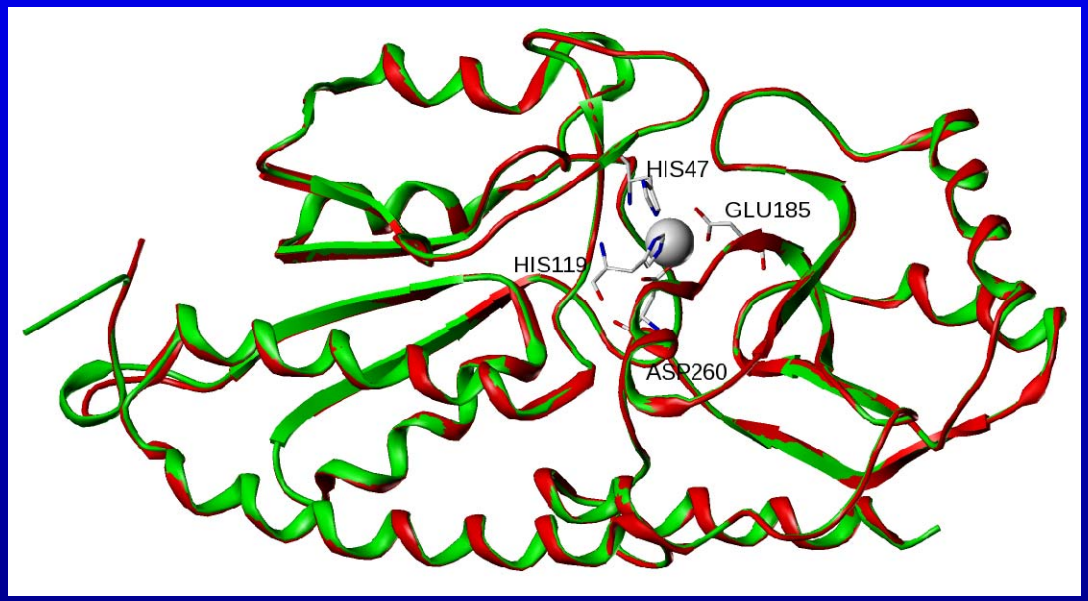


Growth in serum ± 2 μM Mn



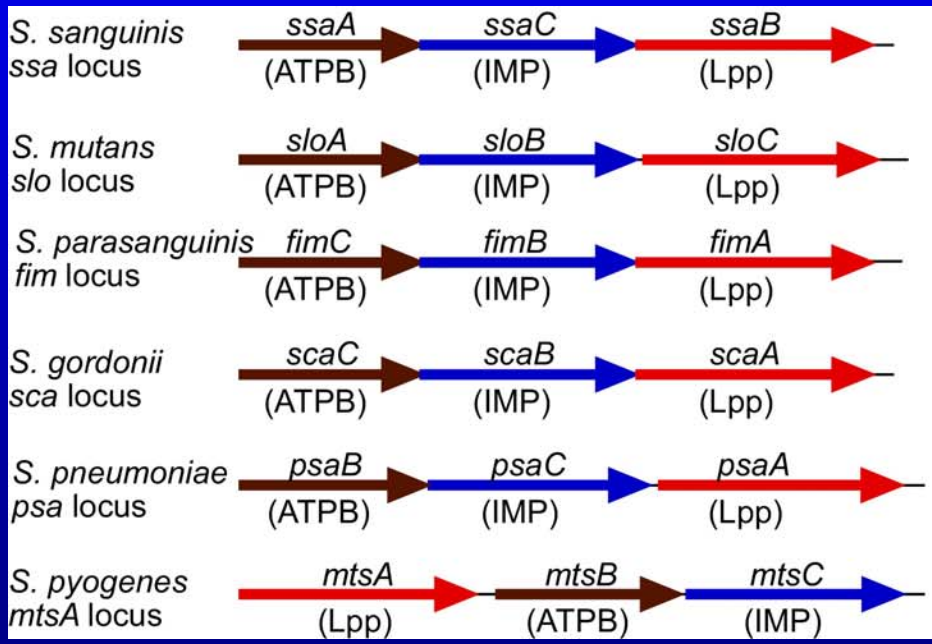
Inhibition of Mn uptake?

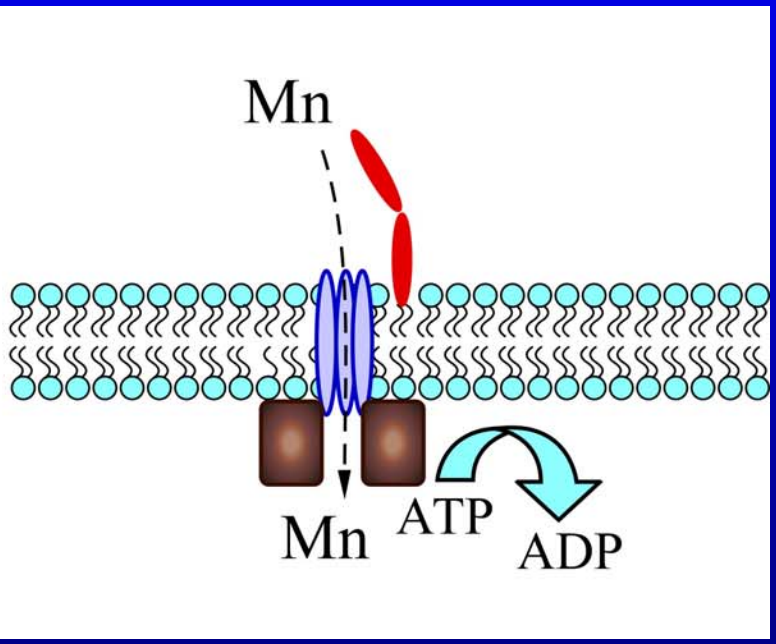
Glen E. Kellogg , Ph.D.
Associate Professor
Department of
Medicinal Chemistry



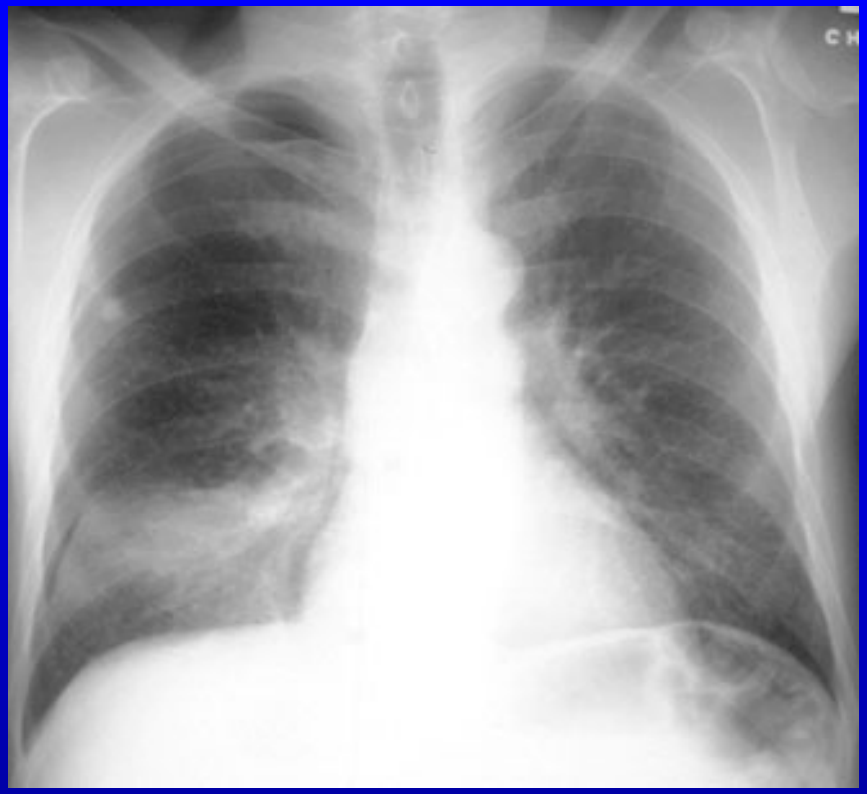
	Saliva	Serum
Mn conc.	36 μM	20 nM

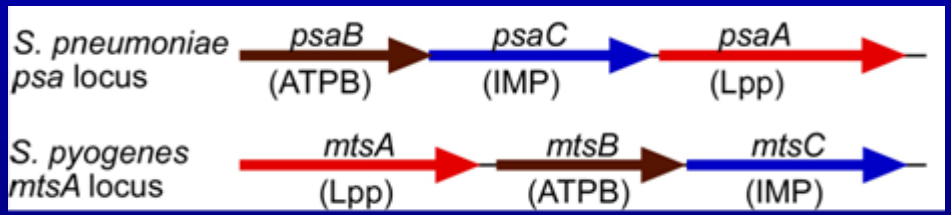
SsaB Family


S. sanguinis
ssa locus
ssaA
(ATPB)
ssaC
(IMP)
ssaB
(Lpp)
S. mutans
slo locus
sloA
(ATPB)
sloB
(IMP)
sloC
(Lpp)
S. parasanguinis
fim locus
fimC
(ATPB)
fimB
(IMP)
fimA
(Lpp)
S. gordonii
sca locus
scaC
(ATPB)
scaB
(IMP)
scaA
(Lpp)
S. pneumoniae
psa locus
psaB
(ATPB)
psaC
(IMP)
psaA
(Lpp)
S. pyogenes
mtsA locus
mtsA
(Lpp)
mtsB
(ATPB)
mtsC
(IMP)


Mn
Mn
ATP
ADP

Other Diseases





Munro and Kitten

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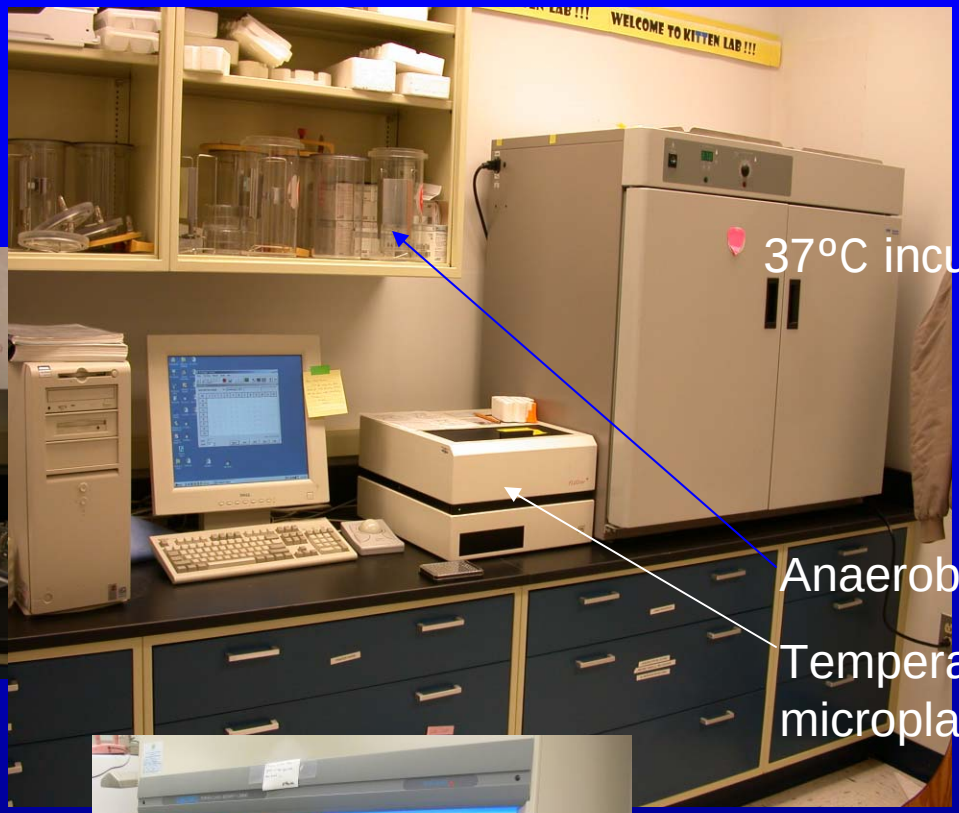
Other Collaborations

- “Low-tech” microbiology

Equipment and Resources

Anoxomat anaerobic system



37°C incubator

Anaerobic jars

Temperature-controlled microplate reader

Biosafety cabinet

Thermal cycler

Other Collaborations

- “Low-tech” microbiology
- Asking neglected questions or old questions in new ways

Other Collaborations

Oral Care and Bacteremia Risk
in Mechanically Ventilated Adults

Deborah J Jones, PhD, MS, RN

Healthcare Acquired Infection Risk and Toothbrush Contamination in the ICU

Michelle Frazelle, PhD, RN, CCRN

- Bacterial species identification
- Epidemiological molecular strain typing

Other Collaborations

- “Low-tech” microbiology
- Asking neglected questions or old questions in new ways
- Asking questions that couldn’t be asked previously

Other Collaborations

- Example:
  - Are particular communities of oral bacteria associated with health or a particular disease?
    - Approach: Metagenomics
    - Method: “Next-generation” deep sequencing

Metagenomics

	HiSeq 2000
Output (2 × 100 bp)	600 Gb
Run Time	~11 days
Cluster Generation	cBot
Paired-end Reads	6 Billion
Single Reads	3 Billion
Maximum Read Length**	2 × 100 bp

GS FLX+ System Performance	
Read Length	Up to 1,000 bp
Mode Read Length	700 bp
Throughput Profile	85% of total bases from reads >500 bp 45% of total bases from reads >700 bp
Typical Throughput	700 Mb
Reads per Run	1,000,000
Consensus Accuracy*	99.997%
Run Time	23 hours

Also Ping Xu, Ph.D., Janina Lewis, Ph.D.

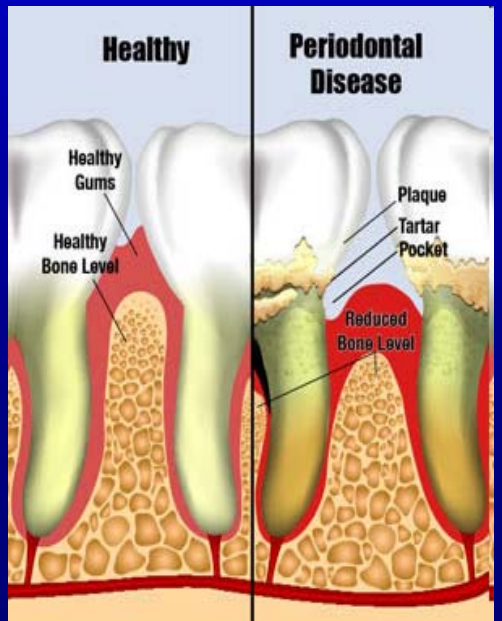
Other Collaborations?

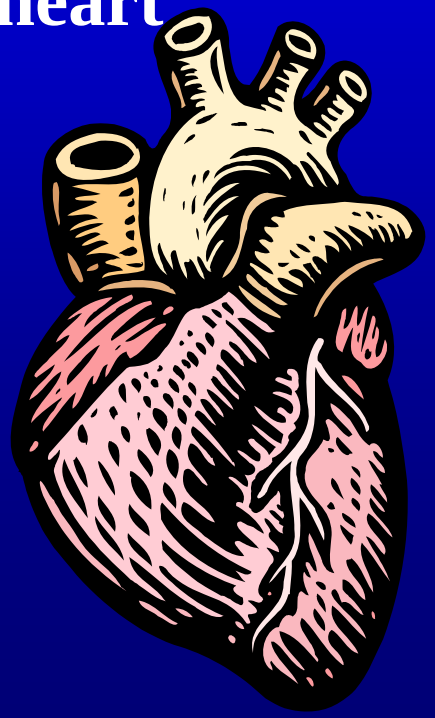
- Alison Montpetit, R.N., Ph.D.
  - Does the oral microflora contribute measurably to the composition of exhaled breath condensate?
  - Could this be useful for diagnostic purposes?

Collaborations Involving Other SOD Researchers Working with Oral Microflora?

Oral Bacteria in Relation to Extra-oral Diseases

- **Infective endocarditis**
- **Periodontal disease** as contributing to **atherosclerosis** and **coronary heart disease**





Periodontal disease

- Direct infection or action at a distance
- Associated with
  - Premature birth or low birth weight
  - Diabetes
  - Others?

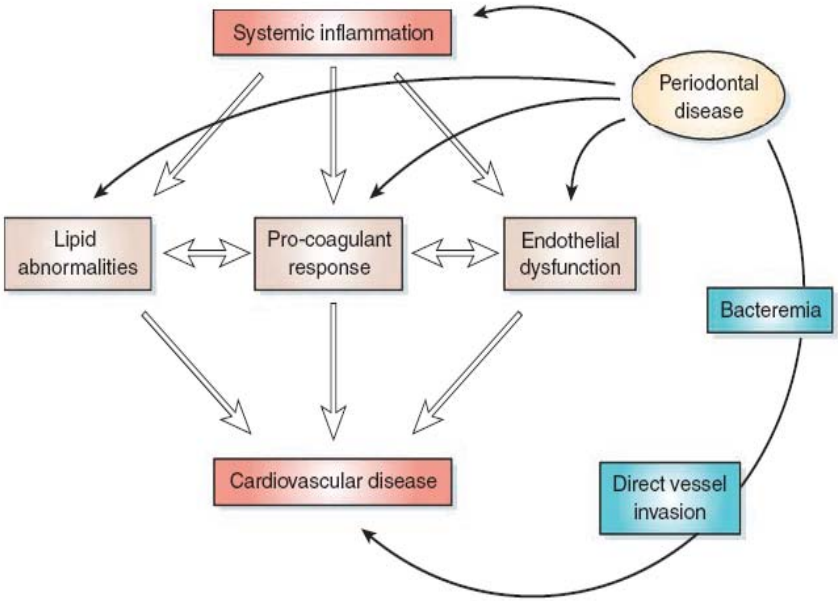


Figure 1 | Impact of periodontal disease in the pathogenesis of cardiovascular disease. The diagram illustrates several mechanisms through which periodontal disease may interact with inflammatory pathway components to promote cardiovascular disease.

Madore, F. 2009. Periodontal disease: a modifiable risk factor for cardiovascular disease in ESRD patients? Kidney Int **75:**672-674.

SOD Faculty Working on Periodontal Disease

Harvey Schenkein, D.D.S., Ph.D.

Esra Sahingur, D.D.S., Ph.D.

Janina Lewis, Ph.D.

What we can do for you

- Provide expertise and equipment for pilot studies involving microbiology
  - "Yes, we can do that!"
- Bring you our ideas for the "next big thing" and be open to yours

What you can do for us

- Bring us your ideas and problems
- Include the SOD in research seminar notices?
- Keep your door open for us (as is already the case)

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